dimers are 4 Å further apart at steady state relative to conventional nucleosomes. These data suggest that CENP-A nucleosomes in solution exist in equilibrium between at least two structural conformations: one that is similar to the conventional nucleosomes and one that is altered by rotation at the CENP-A/CENP-A interface. To further interrogate the structure of CENP-A nucleosomes in solution we are also employing SAXS (small-angle X-ray scattering) and SANS (small-angle neutron scattering). SANS experiments exploit contrast variation schemes to provide information on DNA and protein subunits independently. Our ongoing biophysical studies promise to provide critical insight into how CENP-A nucleosomes distinguish centromeric chromatin in the context of mammalian chromosomes.

2979-Pos Board B134
Two Lysine Residues Enhance the Flexibility of DNA Wrapping around CENP-A Containing Nucleosome Core Particle
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The structural dynamics of two nucleosomes, one was H3 containing and other was CENP-A containing nucleosome, were characterized with molecular dynamics simulations and the findings were experimentally confirmed. The simulations showed that histone proteins of both, which is the core structure of nucleosome, are structurally stable and maintain the structure determined by x-ray crystallography, while the wrapped DNAs are highly flexible at the entry or exit region and largely deviate from the crystal structures. In particular, about 20-25 bp DNAs of entry or exit of the CENP-A containing nucleosomes showed several times of open and close conformational changes within 100ns simulations, which was not observed on the H3 containing nucleosomes. The detailed analysis clarifies that this dynamics difference is due to the difference in two basic amino acids at the α-N helix, two Arg residues of H3 are mutated to Lys residue at the corresponding sites. The difference in ability of forming hydrogen-bond to the DNA controls the flexibility of the nucleosomal DNA at entry or exit region. This increase in flexibility was confirmed with a nuclease susceptibility assay of a nucleosome that contains H3 mutant with the two Arg residues replaced with Lys.

2980-Pos Board B135
Solution Scattering and FRET Studies on Nucleosomes Reveal DNA Unwrapping Effects of H3 and H4 Tails
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Using a combination of small-angle x-ray scattering and fluorescence resonance energy transfer (FRET) measurements we have determined the role of the H3 and H4 histone tails independently in stabilizing nucleosomes and the extent to which the terminal ends of DNA unwrap from the nucleosome core. We have reconstructed solution scattering envelopes for recombinant wild-type, H3 and H4 tail-removed mutants and fit all scattering data with predictions from PDB models. Based on these data combined with FRET results, we found that while all nucleosomes exhibited DNA unwrapping, the extent of this unwrapping was increased for nucleosomes with the H3 tails removed but decreased in nucleosomes with the H4 tails removed. Studies of salt concentration effects showed small (compared to tail effects) but noticeable effects as a function of monovalent ions. These global and local structural data are instrumental in improving our understanding of the role of the H3 and H4 tails and the role of monovalent ions in determining DNA accessibility of nucleosomes.

2981-Pos Board B136
Effect of DNA Methylation on the Assembly of Nucleosomes
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We monitored nucleosome assembly real-time in a time-resolved manner at a single molecule level using FRET. Our data indicate that the mechanical property of nucleosomal DNA altered upon CpG methylation impact on the kinetics of DNA wrapping during nucleosome assembly. Based on the results, we propose paradoxical roles of CpG methylation in gene regulation.

2982-Pos Board B137
Biophysical Characterization of Structural and Energetic Differences between H2A and H2A/H2B Heterodimer Variants from C. elegans
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The compaction of eukaryotic DNA occurs within the nucleus of the cell by the nucleoprotein complex chromatin. Histones, the primary protein components of chromatin, facilitate this packaging to fit full-length DNA into the nucleus. The histones arrange themselves into two H2A/H2B dimers and an H3/H4 tetramer to form a histone octamer. The octamer is surrounded by about 150 base pairs of DNA to form the fundamental repeating unit of chromatin called the nucleosome core particle (NCP). Compaction of DNA facilitated by the NCP is important for restricting access to DNA to regulate DNA-based chemistries including gene expression and replication. Structural changes in chromatin thus act as an essential regulatory mechanism. Differences in nucleosome structure and dynamics play fundamental roles in cell differentiation and in the progression of a variety of diseases. The properties of the nucleosome are often modulated by histone protein variants, specifically H2A variants whose amino acid sequences differ from that of H2A. The work compares how variants of H2A monomer (HTAS-1 and HTZ) alter the secondary structure and stability of the H2A/H2B heterodimer. These variants are known to have different transcription and replication behaviors. We hypothesize that differences in secondary structure and stability of the variants within the H2A/H2B dimer correlate with observed differences in transcription and replication. The secondary structure and thermodynamic stability of H2A variant monomers and the heterodimer composed of H2A variants and H2B were determined using chemical and thermal denaturation monitored with far-UV circular dichroism spectroscopy. This work provides insights into how the physical properties of the histone variants HTAS-1 and HTZ alter the H2A/H2B dimer within the nucleosome.

2983-Pos Board B138
Nucleosome Rewrapping Facilitates Transcription Factor Exchange
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Transcription factors (TFs) play a central role in regulating the transcriptional state of gene. However, TFs are sterically occluded from their target sequences when it is wrapped around the histone octamer into a nucleosome. The combination of nucleosomal DNA unwrapping fluctuations and the high DNA sequence specificity of TFs facilitate TF to binding within nucleosomes. TFs achieve their high DNA sequence specificity by having resident times at their recognition sites that are much longer than minutes. This dramatically limits the rate at which a gene can switch between transcriptionally active and repressed states. We investigated with single-molecule Fluorescence Resonance Energy Transfer(FRET) measurements the influence of nucleosomal DNA wrapping fluctuations on TF binding and release from its target sequence within a mononucleosome and/or a nucleosome array. As expected, we find that nucleosomes inhibit the binding rate of a TF to its target sequence within a nucleosome. In contrast,we observe that the rate of TF dissociation from a nucleosome is increase by over a 100-fold as compared to dissociation from duplex DNA. This result suggests that the nucleosome promotes TF dissociation to facilitate the exchange of TFs at their target sequences and enable rapid regulation of gene expression.

2984-Pos Board B139
New Insights into Nucleosome Unwrapping
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Recently, a new approach for direct mapping of nucleosome centers at base-pair resolution was developed [1] and some intriguing results appeared. About 40% of the inter-dyad distances are smaller than 147 base-pairs, which indicates massive nucleosome unwrapping, genome-wide, in vivo. Paired-end reads give, for the first time, precise information about neighboring nucleosomes which come from the same cell. The histogram of the inter-dyad distances presents small oscillations with the period of about 10 base-pairs. These oscillations indicate a step-wise unwrapping of the nucleosomal DNA from the histone.
One-dimensional lattice models can reproduce characteristic patterns observed in the distribution of the nucleosomes along the chromosomes [2, 3]. We present a statistical mechanics model [4] for the nucleosome unwrapping, which is able to take into account sequence-dependent binding energies, sequence-independent potential barriers and wells, effective two-body interactions between the nucleosomes, competition between different species (e.g. nucleosomes and transcription factors, RNA polymerase, etc.), cooperative-binding, and other important factors which dictate the nucleosome distribution along the DNA. We solve both the direct and the inverse problems.

Using our model, we are able to reproduce the distribution of the inter-dyad distances, which clearly cannot be obtained using the assumption that all histones cover the same length of DNA (i.e. there is no unwrapping).
An extended abstract can be found here: http://www.physics.rutgers.edu/~rchereji/unwrap.pdf
References:
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2985-Pos Board B140
Nucleosome under Torsion
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Nucleosome, the fundamental unit of chromatin, is expected to be affected by torsional stress due to its chiral structure. Here we take advantage of the high spatial and temporal resolution as well as the torque measuring capabilities of an angular optical trap to investigate the impact of torsion on a single nucleosome. We employ buckling assay to study topological transitions in the nucleosome, and stretching assay to probe nucleosome disruption under tension and torque. Our data indicate that torsion differentially affects the stability of the outer and inner turns of nucleosomal DNA. We also observe appearance of an altered nucleosome conformation under moderate positive torque. These findings suggest that torsion can play a facilitatory role during transcription through chromatin.

2986-Pos Board B141
Mechanical Stability of Mononucleosome Revealed by Optical Torque Wrench
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The fundamental chromatin packing unit in eukaryotes is the nucleosome, where ~147 base pairs of DNA are wrapped in ~1.7 turns around a core histone octamer. A crucial question in biology is to explain how proteins are able to access DNA which is tightly bound in chromatin. For example, RNA polymerase must navigate through the nucleosome while transcribing DNA. Hence, the DNA-histone interactions play a key role in gene regulation. Single-molecule force spectroscopy is a powerful tool to probe this system. Prior studies have exerted linear tension to stretch both chromatin fibers and mononucleosome molecules, which have given information on the nature of the free-energy barrier for a particular disruption pathway. We develop a theoretical model including torsional constraints, which suggests that the disruption pathway may be strongly sensitive to the torsional loading of the nucleosome. This is of interest because helicases, polymerases, or other motor proteins may use a combination of force and torque to disrupt chromatin. Experimentally we apply force and torque simultaneously to disrupt a mononucleosome structure using an optical torque wrench. Positive supercoiling is found to destabilize the nucleosome while negative supercoiling has little effect, which is consistent with our model. By determining the influence of supercoiling density on the disruption barrier we obtain more detailed information about DNA-protein interaction strength in nucleosomes.

2987-Pos Board B142
Electrostatic Effects of the Ion Atmosphere on Nucleosome Core Particle Interactions
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Nucleosome-nucleosome attraction is known to be a vital aspect of chromatin compaction. The attraction is primarily electrostatic and is affected by the addition of divalent (or higher valence) ions. The specific mechanisms of this compaction, however, have yet to be determined. In particular there has been recent debate about the role of the nucleosome tails in nucleosome-nucleosome attraction. using buffer equilibrium atomic emission spectroscopy, we have determined the ion composition of the nucleosome in both wildtype and tail-modified mutants under a variety of ionic conditions. These data, when compared to atomic-scale Poisson-Boltzmann equation predictions, will shed light on the electrostatic and tail effects that drive inter-nucleosome interactions in solution.

2988-Pos Board B143
Establishing Real-Time, Single-Molecule Approaches to Study RSC Complex Mediated Nucleosome Remodeling Activities
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Chromatin remodeling complexes utilize the energy of ATP hydrolysis to dynamically alter the histone-DNA interaction and thus regulate the DNA transcription, replication, repair and recombination. yRSC, an essential chromatin remodeling complex in budding yeast belongs to SWI/SNF family remodelers, is shown to possess the ability to alter the nucleosome structure by translocating along DNA or pulling off the histone from nucleosomal DNA. Up to now, many papers have focused on studying nucleosome remodeling process. However, the interaction between RSC and its DNA / nucleosomal substrate and how RSC remodels the nucleosome structure require additional information on its detailed kinetic mechanism. In this study, we combine the single-molecule techniques and traditional biochemical approaches to understand the RSC remodeling activities.

2989-Pos Board B144
The Non-Monotonic Effect of Nucleosome Occupancy on Gene Expression
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Nucleosomes are known to play dynamic role in eukaryotic gene expression. Many inducible genes contain stably positioned nucleosomes in their promoter region. Promoter nucleosomes often hinder access of transcription factors to their regulatory sites on DNA. Accessibility of such regulatory sites by transcription factors requires removal of promoter nucleosomes during transcription activation. Hence, the role of promoter nucleosomes is generally thought to be repressive in gene expression. However, the role of promoter nucleosomes that do not occlude any transcription factor binding sites remains unclear. In present study we varied the stability of non-occluding nucleosome positioned between transcription factor binding site and TATA box region in an inducible yeast promoter. We measured nucleosome stability of promoter nucleosomes by performing in-vivo nucleosome occupancy assay (ChIP), and measured fluorescent protein intensities of downstream gene to quantify gene expression level. We found non-monotonic relationship between nucleosome occupancy and gene expression level, and showed that nucleosome with relatively low stability can lower gene expression level significantly. We present a quantitative model based on the mechanism of nucleosome removal to explain this unexpected effect of a non-occluding nucleosome on gene expression.

2990-Pos Board B145
Nucleosome-Nucleosome Stacking: A Major Element of Chromatin Structure
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Interactions between nucleosome core particles (NCP) are key features of chromatin folding and are inherently related to regulation of DNA replication, transcription and repair. However, little is known about structures of NCP-NCP contacts as well as about the nature and scale of the forces involved. The NCP and chromatin are polyelectrolytes which show a sensitivity of folding and self-association both to ionic environment and to position, sequence and modifications of histone N-termini (tails). Combining experimental and computer modeling approaches, we study nucleosome-nucleosome interaction in both general polyelectrolyte and specific structural context. using native chemical ligation methods, large quantities of H4 histones containing acetylated and methylated lysines were synthesized. The influence of these post-translational modifications on folding and intermolecular association of the NCPs and model chromatin arrays was studied as a function of the nature and concentration of monovalent and multivalent cations (K^+, Na^+, Mg^{2+}, $Co(NH_3)_6^{3+}$, $spermine^{4+}$). Additionally, computational modelling was used to investigate NCP-NCP interaction. A coarse-grained model of the NCP takes into account the details of its structure, based on the single crystal structure at atomic resolution and adequately describes the strong electrostatic forces acting in chromatin by explicit modelling of ions. Most experimental data is well-described by this electrostatic model. However, NCP-NCP close contact (stacking) shows sensitivity to the presence of the H4 histone tail and to acetylation of lysine 16 and other modifications in this histone which is beyond electrostatics. We suggest a structural model explaining the mechanism of these combined modification factors.